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(54) Title: A RETROVIRAL IMMUNOTHERAPY

(57) Abstract: The present inventor has noted that at least two populations of immune cells are produced in response to retroviruses which infect mammals. More particularly, the immune system of a mammal infected with a retrovirus is capable of mounting an immune response against the virus through a group of cells herein generally referred to as "effector cells", however, a second population of cells are also produced which regulate the "effector cells", herein generally referred to as "regulator cells", limiting the mammals' ability to effectively control or eradicate the retroviral infection. Accordingly, the present invention utilizes these observations to provide methods for treating a mammal with a retroviral infection.

A RETROVIRAL IMMUNOTHERAPY

FIELD OF THE INVENTION:

The present invention provides a method of treating a retroviral infection in a mammalian subject. More particularly, the present invention provides a method of treating a retroviral infection which leads to an immunodeficiency-related disease in a human subject.

BACKGROUND OF THE INVENTION:

Human immunodeficiency virus (HIV) induces a persistent and progressive infection leading, in the vast majority of cases, to the development of the acquired immunodeficiency syndrome (AIDS). There are at least two distinct types of HIV: HIV-1 and HIV-2. In humans, HIV infection eventually leads to immune incompetence, opportunistic infections, neurological dysfunctions, neoplastic growth, and ultimately death.

HIV is a member of the lentivirus family of retroviruses. Retroviruses are small enveloped viruses that contain a single-stranded RNA genome, and replicate via the insertion of a DNA intermediate into the host DNA produced by a virally-encoded reverse transcriptase. Other retroviruses include, for example, oncogenic viruses such as human T-cell leukemia viruses (HTLV-I,-II,-III), feline leukemia virus, and the murine type C retroviruses.

The HIV viral particle consists of a viral core, composed in part of capsid proteins designated p24 and p18, together with the viral RNA genome and those enzymes required for early replicative events. Myristylated gag protein forms an outer viral shell around the viral core, which is, in turn, surrounded by a lipid membrane envelope derived from the infected cell membrane. The HIV envelope surface glycoproteins are synthesized as a single 160 kilodalton precursor protein which is cleaved by a cellular protease during viral budding into two glycoproteins, gp41 and gp120. gp41 is a transmembrane glycoprotein and gp120 is an extracellular glycoprotein which remains non-covalently associated with gp41, possibly in a trimeric or multimeric form.

HIV infection is pandemic and HIV-associated diseases represent a major world health problem. Although considerable effort is being put into the design of effective therapeutics, currently no curative anti-retroviral drugs or therapies against AIDS exist. In attempts to develop such drugs, several

stages of the HIV life cycle have been considered as targets for therapeutic intervention (Mitsuya et al., 1991).

Attention has been given to the development of vaccines for the treatment of HIV infection. The HIV-1 envelope proteins (gp160, gp120, gp41) have been shown to be the major antigens for anti-HIV antibodies present in AIDS patients (Barin et al., 1985). Thus far, therefore, these proteins seem to be the most promising candidates to act as antigens for anti-HIV vaccine development. Several groups have begun to use various portions of gp160, gp120, and/or gp41 as immunogenic targets for the host immune system (US 5,141,867; WO 92/22654; WO 91/09872; WO 90/07119; US 6,090,392). Despite these efforts, an effective vaccine strategy for the treatment of HIV infection has not been developed.

The present invention provides an alternate immunotherapy for treating a retroviral infection.

SUMMARY OF THE INVENTION:

The present inventor has noted that at least two populations of immune cells are produced in response to retroviruses which infect mammals. More particularly, the immune system of a mammal infected with a retrovirus is capable of mounting an immune response against the virus through a group of cells herein generally referred to as "effector cells", however, a second population of cells are also produced which regulate the "effector cells", herein generally referred to as "regulator cells", limiting the mammal's ability to effectively control or eradicate the retroviral infection.

Whilst not wishing to be limited by theory, it is proposed that humans contain many sequences which are homologous or near homologous to retroviral sequences fragmented throughout their genome as stable heritable elements. Accordingly, proteins encoded by these sequences may be recognised as "self" during development of the immune response. Subsequent infection by a retrovirus may then also be partially recognized as "self", limiting the immune system's ability to mount a successful immune response. This proposal, at least in part, may explain why up until now it has been difficult to develop an effective vaccine against HIV.

Support for this hypothesis has been provided by Rakowicz-Szulczynska and co-workers (1998 and 2000) who have shown that some antigens associated with breast cancer are molecularly and immunologically

similar to proteins encoded by HIV-1. Similar observations have been made for other cancers. In addition, Coll et al. (1995) have reported antibodies which bind HIV-1 in patients with autoimmune diseases such as Sjogren's syndrome and systemic lupus erythematosus. Furthermore, a BLAST search of the human genome database with the human immunodeficiency virus genome sequences indicates many regions of significant identity between the two genomes.

It has also been noted that the relative number of effector cells expand in response to an antigen before the regulator cells expand. This provides an opportunity to prevent the production of, or destroy, the "regulator cells" whilst maintaining the "effector cells".

Accordingly, in a first aspect the present invention provides a method of treating a retroviral infection in a mammalian subject, the method comprising administering to the subject a composition which increases the number of, and/or activates, effector cells directed against the retrovirus, and subsequently administering to the subject an agent which inhibits the production of, and/or destroys, regulator cells, wherein the timing of administration of the agent is selected such that the activity of the effector cells is not significantly reduced.

There are many ways in which the number, and/or activity, of effector cells directed against a retrovirus can be increased. In some instances, this will occur by an inadvertent infection with the retrovirus, for example, a needle prick of a syringe containing the virus. As is well known, health workers and researchers run the risk of viral infection through needle stick injury. Similarly, the general public are also exposed to this danger through discarded syringes, particularly on the beach, and muggings with such syringes. Therefore, upon suspected exposure to a retrovirus, particularly HIV, the mammalian subject could utilize the method of the present invention.

The method of the present invention can also be used to treat a mammalian subject which has been infected with a retrovirus for some time. Although the immune system of such a subject has already been exposed to the retrovirus, the addition of further retroviral antigens may lead to a further effector cell response with subsequent opportunity to ablate the regulators of these new effectors.

Subjects infected with a retrovirus can be treated with antiretroviral drugs to keep the viral load low. Such subjects also could be administered with the composition to initiate a new effector cell immune response whilst the subsequent administration of the agent would ablate regulator cells.

In a preferred embodiment of the first aspect, the agent is administered approximately when CD8+CD4- T cell numbers have peaked in response to the administration of the composition.

Further, it is preferred that the agent is administered approximately when the number of viral particles has begun to stabilize or increase following administration of the composition.

In yet another preferred embodiment of the first aspect, restimulation of effector cells in an infected mammalian subject can be achieved by a composition which comprises a retroviral antigenic polypeptide. Preferably, the antigenic polypeptide is provided to the subject by administering a vaccine comprising the retrovirus antigenic polypeptide and a pharmaceutically acceptable carrier. More preferably, the vaccine further comprises an adjuvant.

In another embodiment the antigenic polypeptide is provided to the subject by administering a DNA vaccine encoding the retroviral antigenic polypeptide.

In yet another embodiment, the antigenic polypeptide is provided to the subject by the consumption of a transgenic plant expressing the retroviral antigenic polypeptide.

Withdrawal of antiretroviral treatment typically causes a rapid re-expansion of effector cells against the re-emergent virus in an infected subject. Accordingly, at the appropriate time the agent can be administered to ablate the regulator cells without the need to administer a composition as defined herein.

Therefore, in a second aspect the present invention provides a method of treating a retroviral infection in a mammalian subject, the method comprising exposing the subject to antiretroviral drug therapy, and subsequently administering to the subject an agent which inhibits the production of, and/or destroys, regulator cells, wherein the agent is administered after the antiretroviral drug therapy has concluded and a resulting expansion in retroviral numbers has led to an increase in the number, and/or activation, of effector cells directed against the retrovirus, and

wherein the timing of administration of the agent is selected such that the activity of effector cells is not significantly reduced.

In a preferred embodiment of the second aspect, the agent is administered approximately when CD8+CD4- T cell numbers have peaked in response to the conclusion of the antiretroviral drug therapy.

Upon removal of antiretroviral drug therapy the viral load increases (for example, see Daar et al., 1998). This results in an expansion and/or activation of effector cells directed against the retrovirus which begin to stabilize the viral load. It is approximately when the retroviral numbers have peaked, or begun to decrease after this peak, that the agent should be administered.

Accordingly, in a further preferred embodiment of the second aspect, the agent is administered approximately when the number of viral particles has peaked, or begun to decrease after this peak, in response to the conclusion of the antiretroviral drug therapy.

Preferably, the agent used in regulator cell ablation is selected from the group consisting of anti-proliferative drugs, radiation, and an antibody which binds specifically to CD4+ T cells. Preferably, the anti-proliferative drug is selected from the group consisting of vinblastine and anhydro vinblastine.

Preferably, the retrovirus is selected from the group consisting of HIV-1, HIV-2, HTLV-1 and HTLV-2.

As would be readily appreciated by those skilled in the art, the methods of the present invention may be repeated to provide a more complete treatment.

Preferably, the mammalian subject is a human.

In a third aspect, the present invention provides for the use of an agent which inhibits the production of, and/or destroys, regulator cells in the manufacture of a medicament for treatment of a retroviral infection in a mammalian subject.

In a preferred embodiment of the third aspect, the agent is selected from the group consisting of anti-proliferative drugs, and an antibody which binds specifically to CD4+ T cells.

In each aspect outlined above, ablation of the "regulator" population allows the "effector" population to reduce or eradicate the retroviral load as any down regulation of effectors (due to self-tolerance mechanisms) has been removed.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The invention will hereinafter be described by way of the following non-limiting Figures and Examples.

BRIEF DESCRIPTION OF THE DRAWINGS:

Figure 1: Time course of MAIDS in B6 mice infected with LP-BM5.

Figure 2: Effect of a single dose of vinblastine (6mg/kg i.p.) on MAIDS progression at 10 weeks post infection.

Figure 3: Spleen histology of vinblastine treated mice 10 weeks post infection.

Figure 4: Protection from MAIDS at 20 weeks post infection following vinblastine therapy. n=5.

Figure 5: Rechallenge with MAIDS virus following protective vinblastine therapy. n=5.

Figure 6: Effect of day 14 vinblastine on spleen cell percentages in MAIDS infected (MAIDS+) and control mice (MAIDS-). n=3.

Figure 7: Spleen transfer experimental protocol.

Figure 8: Spleen cell transfers from MAIDS infected donor mice. n=7.

Figure 9: *In vivo* depletion experimental protocol.

Figure 10. *In vivo* depletion of CD4+ or CD8+ cells at day 14 post infection. n=5.

DETAILED DESCRIPTION OF THE INVENTION:

Immune Response to Retroviral Infection

As used herein the term "treating" or "treat" means a reduction in retroviral load is achieved. Most preferably, the retroviral load is completely eradicated.

Although the precise nature of the "regulator cells" has not yet been determined it has been established that these cells include a subpopulation of CD4+ cells.

CD4+ cells express the marker known in the art as CD4. Typically, the term "CD4+ T cells" as used herein does not refer to cells which also express

CD8. However, this term can include T cells which also express other antigenic markers such as CD25.

Although the precise nature of the "effector cells" has not yet been determined, it is known that these cells include the T cell population known as CD8+ cells.

As used herein, the term "ablate" or "ablation" when referring to the exposure of the "regulator cells" to the agent means that the number, and/or activity, of regulator cells is down-regulated by the agent. Most preferably, the number, and/or activity, of regulator cells is completely eradicated by the agent.

Agents which Inhibit the Production of, and/or Destroy, Regulator Cells

The agent can be any factor or treatment which selectively or non-selectively results in the destruction, or the inhibition of the production, of regulator cells. For example, a CD4+ specific antibody could be used to specifically target CD4+ T cells. However, in some instances a non-selective agent could be used, such as an anti-proliferative drug or radiation, both of which destroy dividing cells.

The term "anti-proliferative drug" is a term well understood in the art and refers to any compound that destroys dividing cells or inhibits them from undergoing further proliferation. Anti-proliferative drugs include, mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, hexamethyl-melamine, thiotepa, busulfan, carmustine, lomustine, semustine, streptozocin, dacarbazine, methotrexate, fluorouracil, floxuridine, cytarabine, mercaptopurine, thioguanine, pentostatin, vinblastine, anhydro vinblastine, vincristine, etoposide, teniposide, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, mitomycin, L-asparaginase, cisplatin, mitoxantrone, hydroxyurea, procarbazine, mitotane, aminoglutethimide, prednisone, hydroxyprogesterone caproate, medroprogesterone acetate, megestrol acetate, diethylstilbestrol, ethinyl estradiol, tamoxifen, testosterone propionate, radioactive isotopes, ricin A chain, taxol, diphtheria toxin and pseudomonas exotoxin A.

Timing of Exposing the Subject to the Agent

As outlined above, the present invention relies on the observation that the relative number of effector cells expands in response to an antigen before

the regulator cells. Accordingly, as used herein, the term "the activity of the effector cells is not significantly reduced" means that the timing of the administration of the agent is such that the agent exerts a proportionally greater effect against the regulator cells than the effector cells. It is clearly preferred that the agent is administered at a time where the ratio of effect against the regulator cells to the effect against effector cells is greatest.

It has been reported that after the initial fall in viral load following anti-retroviral treatment, for instance in subjects infected with HIV, there is an increase in viral load and a subsequent stimulation of the immune response to the increase in viral load upon withdrawal of treatment (Oritz et al., 1999; Kilby et al., 2000; Lifson et al., 2000). Accordingly, the exposure of the subject to anti-viral therapy followed by removal of the therapy could be used to increase the number of, and/or activate, effector cells directed against the retrovirus enabling a targetable regulator cell expansion.

An example of the appropriate time for administering the agent can be determined by reference to Daar et al. (1998). This document provides the viral load, and CD8+ and CD4+ T cell levels, from a patient who is taken off highly active antiretroviral therapy (also known as HAART). After the initial rise in viral load, a reduction in viral load (shoulder at day ~225) occurs when the CD8+ T cells have reached maximum numbers, and therefore effect (see Figure 1 of Daar et al., 1998). Immediately beyond this time point (a few days) CD8+ T cells start to drop off and viral load begins to stabilize after the decline. This point (the peaks) could be used as an inference point to predict the clonal expansion of the subsequent regulator cells and therefore an intervention point for regulator cell ablation. It is where the viral load stops declining that the regulator cells have down regulated the effector cells, but it is before this point that the agent should be applied, namely when the majority of regulators are in clonal expansion (mitosis).

Techniques known in the art can be used to monitor the growing population of regulator cells following the stimulation of an immune response.

Serial blood samples can be collected and quantitatively screened for all CD4+ subsets by FACS analysis. This FACS monitoring will need to be maintained until the regulator cells begin clonally expanding in response to the stimulus. In most instances, this time point will need to be empirically determined in subjects at different stages of infection as their immune

response kinetics may vary. Other possible assays include lymphocyte proliferation/activation assays and various cytokine level assays (for example an assay for IL-6).

Another avenue of determining the time point for administering the agent is to monitor the viral load following stimulation of the immune response. It is envisaged that the viral load decreases due to the activity of the effector cells, however, the subsequent increase in regulator cells would down-regulate the effector cells resulting in a slowing of the viral load decrease. Accordingly, the agent could be administered prior to the slowing of the decrease in viral load. Techniques known in the art, for example RT-PCR, could be used to monitor viral load in these circumstances.

Monitoring may need to be very frequent, for example every few hours, to ensure the correct time point is selected for administration of the agent.

Optimally, the monitoring is continued to determine the affect of the agent. Insufficient ablation, re-emergence of the regulator cells or increases in viral load within, for example, about 7 days of treatment will mean that the method of the present invention should be repeated. Such repeated cycles of treatment may generate immunological memory. It is therefore possible that the present invention, used in repetitive mode, may provide some prophylactic protective effect.

Antigenic Polypeptides

As used herein, an "antigenic" polypeptide is any polypeptide sequence that contains an epitope which is capable of producing an immune response against the retrovirus. Typically, the antigenic polypeptide will comprise a sequence which is highly conserved in most retroviral isolates. However, it is envisaged that a particular retrovirus infecting an individual could be characterized and an antigenic polypeptide produced which matches the sequences of the isolate to maximise the possibility of an effective immune response.

Information regarding HIV antigens such as gp120 and other candidates can be found in Stott et al (1998).

The antigenic polypeptides can be provided in any manner known in the art which leads to an immune response. Antigenic polypeptides can be, for example, native, recombinant or synthetic. Such antigenic polypeptides include, but are not limited to, viral proteins that are responsible for

attachment to cell surface receptors to initiate the infection process such as envelope glycoproteins.

Native antigenic polypeptides can be prepared, for example, by providing attenuated retrovirus, heat inactivated retrovirus or any other killed retrovirus.

The antigenic polypeptides can be provided as isolated polypeptides in a vaccine composition. In this instance the polypeptide can be purified from retroviral infected cells, expressed and isolated from recombinant cells, or synthetically produced using a peptide synthesizer.

Unless otherwise indicated, the recombinant DNA techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) and are incorporated herein by reference.

Vaccines

Vaccines may be prepared from one or more retroviral polypeptides. The preparation of vaccines which contain an antigenic polypeptide is known to one skilled in the art. Typically, such vaccines are prepared as injectables, or orals, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection or oral consumption may also be prepared. The preparation may also be emulsified, or the protein encapsulated in liposomes. The antigenic polypeptides are often mixed with carriers/excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable carriers/excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof.

In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine.

As used herein, the term "adjuvant" means a substance that non-specifically enhances the immune response to an antigenic polypeptide. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. Further examples of adjuvants include aluminum hydroxide, aluminum phosphate, aluminum potassium sulfate (alum), bacterial endotoxin, lipid X, *Corynebacterium parvum* (*Propionobacterium acnes*), *Bordetella pertussis*, polyribonucleotides, sodium alginate, lanolin, lysolecithin, vitamin A, saponin, liposomes, levamisole, DEAE-dextran, blocked copolymers or other synthetic adjuvants. Such adjuvants are available commercially from various sources, for example, Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.) or Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Michigan).

The proportion of antigenic polypeptide and adjuvant can be varied over a broad range so long as both are present in effective amounts. For example, aluminum hydroxide can be present in an amount of about 0.5% of the vaccine mixture (Al_2O_3 basis). Conveniently, the vaccines are formulated to contain a final concentration of antigenic polypeptide in the range of from 0.2 to 200 μg/ml, preferably 5 to 50 μg/ml, most preferably 15 μg/ml.

After formulation, the vaccine may be incorporated into a sterile container which is then sealed and stored at a low temperature, for example 4°C, or it may be freeze-dried. Lyophilisation permits long-term storage in a stabilised form.

The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories,

traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1% to 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% to 95% of active ingredient, preferably 25% to 70%. Where the vaccine composition is lyophilised, the lyophilised material may be reconstituted prior to administration, e.g. as a suspension. Reconstitution is preferably effected in buffer.

Capsules, tablets and pills for oral administration to a patient may be provided with an enteric coating comprising, for example, Eudragit "S", Eudragit "L", cellulose acetate, cellulose acetate phthalate or hydroxypropylmethyl cellulose.

DNA Vaccination

DNA vaccination involves the direct *in vivo* introduction of DNA encoding an antigen into tissues of a subject for expression of the antigen by the cells of the subject's tissue. Such vaccines are termed herein "DNA vaccines" or "nucleic acid-based vaccines". DNA vaccines are described in US 5,939,400, US 6,110,898, WO 95/20660 and WO 93/19183, the disclosures of which are hereby incorporated by reference in their entireties. The ability of directly injected DNA that encodes an antigen to elicit a protective immune response has been demonstrated in numerous experimental systems (see, for example, Conry et al., 1994; Cardoso *et al.*, 1996; Cox et al., 1993; Davis et al.,1993; Sedegah et al., 1994; Montgomery et al., 1993; Ulmer et al., 1993; Wang et al., 1993; Xiang et al., 1994; Yang et al., 1997).

To date, most DNA vaccines in mammalian systems have relied upon viral promoters derived from cytomegalovirus (CMV). These have had good efficiency in both muscle and skin inoculation in a number of mammalian species. A factor known to affect the immune response elicited by DNA immunization is the method of DNA delivery, for example, parenteral routes can yield low rates of gene transfer and produce considerable variability of gene expression (Montgomery et al., 1993). High-velocity inoculation of

plasmids, using a gene-gun, enhanced the immune responses of mice (Fynan et al., 1993; Eisenbraun et al., 1993), presumably because of a greater efficiency of DNA transfection and more effective antigen presentation by dendritic cells. Vectors containing the nucleic acid-based vaccine of the invention may also be introduced into the desired host by other methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), or a DNA vector transporter.

Vaccines Derived from Transgenic Plants

Transgenic plants producing a retroviral antigenic polypeptide can be constructed using procedures well known in the art. A number of plant-derived edible vaccines are currently being developed for both animal and human pathogens (Hood and Jilka, 1999). Immune responses have also resulted from oral immunization with transgenic plants producing virus-like particles (VLPs), or chimeric plant viruses displaying antigenic epitopes (Mason *et al.*, 1996; Modelska *et al.*, 1998; Kapustra *et al.*, 1999; Brennan *et al.*, 1999). It has been suggested that the particulate form of these VLPs or chimeric viruses may result in greater stability of the antigen in the stomach, effectively increasing the amount of antigen available for uptake in the gut (Mason *et al.* 1996, Modelska *et al.* 1998).

EXAMPLES

In order to demonstrate the present invention a murine AIDS model was used.

A murine AIDS (MAIDS) pathology induced by LP-BM5 murine leukemia virus (MuLV) in susceptible mice is an effective tool to investigate mechanisms of retrovirus-induced immunodeficiency. The MAIDS animal model displays a number of features of human AIDS. Infection of a susceptible strain such as C57BL/6 mice with LP-BM5 leads to chronic splenomegaly, hypergammaglobulinaemia and development of immunodeficiency in both T and B cells. *In vitro*, there is a progressive impairment in the responsiveness of T-cells and B-cells to mitogenic or specific antigenic stimuli. *In vivo*, infected mice become increasingly susceptible to challenge with a variety of opportunistic organisms and can

develop B-cell lymphoma. Deaths are first observed at 8-10 weeks post-infection (pi), and all mice die within 24 weeks (Figure 1). These alterations in immune function reflect complex changes in the phenotype and function of all components of the immune network.

1. Therapeutic Effects of a Single Dose of Vinblastine Administered at Different Times Post Infection

The treatment with a single dose of the anti-mitotic agent vinblastine (Vb) at different times pi is able to prevent the development/progression of MAIDS. Figure 2 shows the effect on MAIDS development (represented by average spleen weight at 10 weeks pi) of a single 6mg/kg i.p. dose of Vb given at various times pi ranging from 6hr to 28 days. Clearly, the Vb treatment is remarkably therapeutic when administered at 6 hr or 14 days pi with 100% (10/10) and 74% (20/27) of mice, respectively, showing no signs of MAIDS development at 10 weeks pi (as determined by spleen weight, histology and FACS analysis). A similar protective effect was observed in some mice treated at 6 days (6/13 or 46%) pi. Although not as pronounced as the protection seen after day 14 treatment, moderate protection from MAIDS development was still observed. However, it is also important to note that treatment at many other time points, such as day 2 and day 7 pi, resulted in no protection against MAIDS development. The total protection from MAIDS development seen in mice given Vb at 6 hrs pi is not unexpected as the virus replicates in actively replicating cells which are the target of the anti-mitotic drug thus any cells infected with the virus are killed by the Vb preventing the virus from rapidly establishing an infection in the host. However, the highly protective effect observed after a single treatment with Vb at 14 days pi is quite remarkable as, by this stage, the viral infection is well established and the early disease processes are well underway. We propose that this highly therapeutic effect may be due to the Vb targeting a particular subset of immune regulator cells during their expansion phase and thereby altering the immune response to the viral infection. It is hypothesised that the down-regulation of immune effector cells is removed by the Vb treatment targeting the regulator cells, thus allowing more effective and possibly even complete clearance of the virus by the effector cells of the immune system.

2. Further Characterisation of the Day 14 Therapeutic Effect

2.1. Spleen histology in Vb treated mice at 10 weeks post infection

Spleens from the Day 14 pi Vb treated mice as well as infected and uninfected control mice were weighed and examined histologically. The splenic architecture of all MAIDS infected mice was profoundly disorganised as previously reported (Hartley *et al.*, 1989). In contrast, the splenic architecture of the Vb treated mice with normal spleen weights (below 0.25g) was indistinguishable from that of uninfected control mice (Figure 3).

In support of this histological data, preliminary results based on FACS analysis of spleen cells from day 14 pi Vb treated mice at 10 weeks pi showed cellular proportions ($CD4^+$, $CD8^+$ and B cells) and distributions like those observed in normal uninfected mice (data not shown).

2.2. Long-term protection from MAIDS development following Vb therapy

In order to determine if the infected mice were fully protected from MAIDS development or if the Vb treatment was merely delaying the disease onset and progression we treated mice with Vb at day 14 pi and waited until 20 weeks pi to examine the mice. The results of this experiment (Figure 4) showed that 80% (4/5 mice) were protected from any splenomegaly at 20 weeks pi confirming the highly effective action of the day 14 Vb treatment regime.

2.3. No protection from virus rechallenge following Vb therapy

To determine if mice protected from MAIDS by the day 14 pi Vb treatment develop an immune response which would then protect them from a subsequent rechallenge with the MAIDS virus groups of mice, given the Vb therapy at day 14 pi, were rechallenged with the MAIDS virus at 3 or 8 weeks post Vb treatment. The mice were not protected from viral rechallenge and developed splenomegaly and lymphadenopathy indicating MAIDS development at 20 weeks (Figure 5). This is not an unexpected result as the dominant immunosuppression by the regulator population of cells would have been restored as a consequence of the immune effector response to the second (untreated) infection.

3. Vb therapy targets $CD4^+$ cells

3.1. Direct FACS Analysis of Spleen Cells Following Vb Therapy on Day 14 Post Infection

FACS analysis of $CD4^+$and $CD8^+$ T cells prepared from spleens of mice treated with Vb on Day 14 pi was performed (Figure 6). $CD25^+CD4^+$ T cells were also examined as they have recently been identified as having an important regulatory function in mouse models of autoimmune disease and tumour immunology (Takahashi *et al.*, 1998; Shimizu et al., 2000). It is possible that these cells may play a role in the regulation of the immune response to MAIDS. Day 14 pi and uninfected control mice were given Vb therapy and the spleens collected at 48 hours post Vb treatment. Analysis of the data showed that while all cell subsets are reduced by the Vb therapy, comparison of the uninfected to infected ratios showed that it is the $CD4^+$ T cells and $CD25^+CD4^+$ T cells that are preferentially targeted by the Vb treatment in MAIDS infected mice.

3.2. Spleen cell transfer experiments: Vb therapy targets $CD4^+$ cells

The previous experiment showed that major immune cell subsets ($CD4^+$ and $CD8^+$ cells) resident in the spleen are all affected by Vb treatment, however, it appears that $CD4^+$ cells are preferentially targeted indicating that a subset of these cells may be clonally expanding at day 14 pi. The experiments illustrated in Figure 7 were designed to determine if a single dose of Vb, administered at 14 days pi, is eliminating a population of expanding $CD4^+$ regulator T cells and thereby releasing effector cells from down-regulation and clearing the host of the MAIDS virus.

Mice were infected with MAIDS, received day 14 pi Vb treatment and then received an adoptive transfer of $\sim 10^7$spleen cells from donor mice prepared as outlined in Figure 7. For each experiment [designated (I), (II) and (III)] groups consisted of 7 recipient mice and 9 donor mice. Mice in Group I received whole spleens from infected mice that had not received any Vb treatment. Mice in Group II received whole spleen cell preparations, from infected mice, that had been depleted of $CD4^+$ cells using a FACS cell sorter after staining with fluorochrome-labeled anti-CD4 monoclonal antibody. Cell sorting resulted in removal of 99.5% of $CD4^+$ cells from the spleen cell preparations. Mice in Group III received whole spleens from infected donor mice that also received Vb treatment on day 14 pi.

Infusing Vb-treated MAIDS infected mice with spleen cells from MAIDS infected donors completely prevented Vb from protecting mice from MAIDS development (Figure 8). Moreover, the spleen cells that overcame the protective effect of Vb were $CD4^+$ cells because when $CD4^+$ cells were removed from the donor spleen cells prior to transfer, by FACS sorting, the protective effect of Vb was not overcome. In addition, infusion of Vb-treated MAIDS infected mice with spleen cells from MAIDS infected donor mice that had also been given day 14 Vb showed these mice to be fully protected from MAIDS development (Figure 8).

3.3. In vivo *depletion of $CD4^+$ cells at day 14 post infection results in protection from MAIDS development*

The spleen transfer results are consistent with the interpretation that, at day 14 pi, immune effector cells co-exist with a clonally expanding population of regulator cells which are $CD4^+$, and that Vb is therapeutic by virtue of its ability to destroy the latter. Therefore, an *in vivo* depletion of $CD4^+$ cells at the 14 day pi time point should mimic the effect of Vb. A flow diagram of the experiment is presented in Figure 9. Groups of 5 mice were infected with the MAIDS virus and then treated with monoclonal antibodies to deplete the host of either the $CD4^+$ or $CD8^+$ T cell subset at day 14 pi. Monoclonal antibodies were collected from the supernatants of antibody-producing hybridoma cell lines and purified by ammonium sulphate precipitation. Testing *in vivo* resulted in a 98% reduction in $CD4^+$ cells and a 95% reduction in $CD8^+$ cells from a single injection (0.5mg i.p.) of the appropriate concentrated monoclonal antibody preparation.

Clearly, treatment at day 14 pi with the anti-$CD4^+$ monoclonal antibody resulted in prevention of MAIDS development in infected mice thereby mimicking the effect of a Vb injection at day 14 pi (Figure 10). In contrast, *in vivo* depletion of $CD8^+$ cells at day 14 pi had no effect on disease progression. This result further supports the hypothesis that a population of $CD4^+$ T cells is functionally down-regulating immune effector cells responding to the viral infection. In addition, the removal of the dominant $CD4^+$ regulator cells enables the immune effector cells to respond more effectively to the viral infection resulting in far slower disease progression or perhaps complete clearance of the MAIDS virus from its host.

4. Summary

The present examples indicate that there is a population of "regulator" cells that down regulate the immune response to the MAIDS virus, allowing the virus to proliferate and cause disease. Removal of the regulator cells at the appropriate times after infection allows for effector cells (such as B cells and $CD8^+$ T cells) to more effectively clear the virus, preventing the development of disease. These regulator cells are proposed to control the activation and/or function of cells such as $CD8^+$ T cells and B cells, which act as effector cells, to fight viral infection.

Modulation of the immune response by specifically eliminating a population of immune regulator cells has previously been investigated by Robert North and colleagues using a murine T cell lymphoma model (North and Awwad, 1990). North proposed that the immunogenic tumour was able to establish and grow due to down regulation of the immune response by a population of $CD4^+$ regulator T cells. This regulation did not allow the immune response to develop sufficiently in magnitude to cause tumour regression. It was noted that treatment with a single dose of Vinblastine (Vb) at different times following tumour inoculation resulted in enhancement (day 10) or regression (days 4, 6, 13 and 15) of the tumour. North hypothesised that the observed regression was due to elimination of the $CD4^+$ regulator T cells at those time points. In a series of experiments involving the selective depletion of $CD4^+$ and $CD8^+$ T cells the regulator cells were identified as being of the $CD4^+$ phenotype.

The chromosomal DNA of inbred and wild mice contains multiple copies of sequences reactive with MuLV nucleic acid probes. Among these sequences are complete, potentially infectious genomes of numerous MuLVs (Chattopadhyay *et al.*, 1980). All MuLVs share high sequence homology. We hypothesize that when these endogenous MuLV proteins are expressed during development they are recognised as self-antigens by the immune system. Due to the high level of homology between endogenous and exogenous MuLV nucleic and amino acid sequences an infecting MuLV may also be partly recognised as self by the immune system, and any immune response to the viral infection will be down-regulated, resulting in a lack of viral clearance and the development of disease.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

All publications discussed above are incorporated herein in their entirety.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed, particularly in Australia, before the priority date of each claim of this application.

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CLAIMS:

1. A method of treating a retroviral infection in a mammalian subject, the method comprising administering to the subject a composition which increases the number of, and/or activates, effector cells directed against the retrovirus, and subsequently administering to the subject an agent which inhibits the production of, and/or destroys, regulator cells, wherein the timing of administration of the agent is selected such that the activity of the effector cells is not significantly reduced.

2. The method of claim 1, wherein the agent is administered approximately when CD8+CD4- T cell numbers have peaked in response to the administration of the composition.

3. The method of claim 1, wherein the agent is administered approximately when the number of viral particles has begun to stabilize or increase following administration of the composition.

4. The method according to any one of claims 1 to 3, wherein the composition comprises a retroviral antigenic polypeptide.

5. The method of claim 4, wherein the retroviral antigenic polypeptide is provided to the subject by administering a vaccine comprising the retrovirus antigenic polypeptide and a pharmaceutically acceptable carrier.

6. The method of claim 5, wherein the vaccine further comprises an adjuvant.

7. The method of claim 4, wherein the antigenic polypeptide is provided to the subject by administering a DNA vaccine encoding the retroviral antigenic polypeptide.

8. The method of claim 4, wherein the antigenic polypeptide is provided to the subject by the consumption of a transgenic plant expressing the retroviral antigenic polypeptide.

9. The method according to any one of claims 1 to 8, wherein the subject has been exposed to antiretroviral drug therapy before the composition is administered.

10. A method of treating a retroviral infection in a mammalian subject, the method comprising exposing the subject to antiretroviral drug therapy, and subsequently administering to the subject an agent which inhibits the production of, and/or destroys, regulator cells, wherein the agent is administered after the antiretroviral drug therapy has concluded and a resulting expansion in retroviral numbers has led to an increase in the number, and/or activation, of effector cells directed against the retrovirus, and wherein the timing of administration of the agent is selected such that the activity of effector cells is not significantly reduced.

11. The method of claim 10, wherein the agent is administered approximately when CD8+CD4- T cell numbers have peaked in response to the conclusion of the antiretroviral drug therapy.

12. The method of claim 10, wherein the agent is administered approximately when the number of viral particles has peaked, or begun to decrease after this peak, in response to the conclusion of the antiretroviral drug therapy.

13. The method according to any one of claims 1 to 12, wherein the agent is selected from the group consisting of anti-proliferative drugs, radiation, and an antibody which binds specifically to CD4+ T cells.

14. The method according to claim 13, wherein the anti-proliferative drug is selected from the group consisting of vinblastine and anhydro vinblastine.

15. The method of according to any one of claims 1 to 14, wherein the retrovirus is selected from the group consisting of HIV-1, HIV-2, HTLV-1 and HTLV-2.

16. The method according to any one of claims 1 to 15, wherein the method is repeated at least once.

17. The method according to any one of claims 1 to 16, wherein the mammalian subject is a human.

18. Use of an agent which inhibits the production of, and/or destroys, regulator cells in the manufacture of a medicament for treatment of a retroviral infection in a mammalian subject.

19. The use according to claim 18, wherein the agent is selected from the group consisting of anti-proliferative drugs, and an antibody which binds specifically to CD4+ T cells.

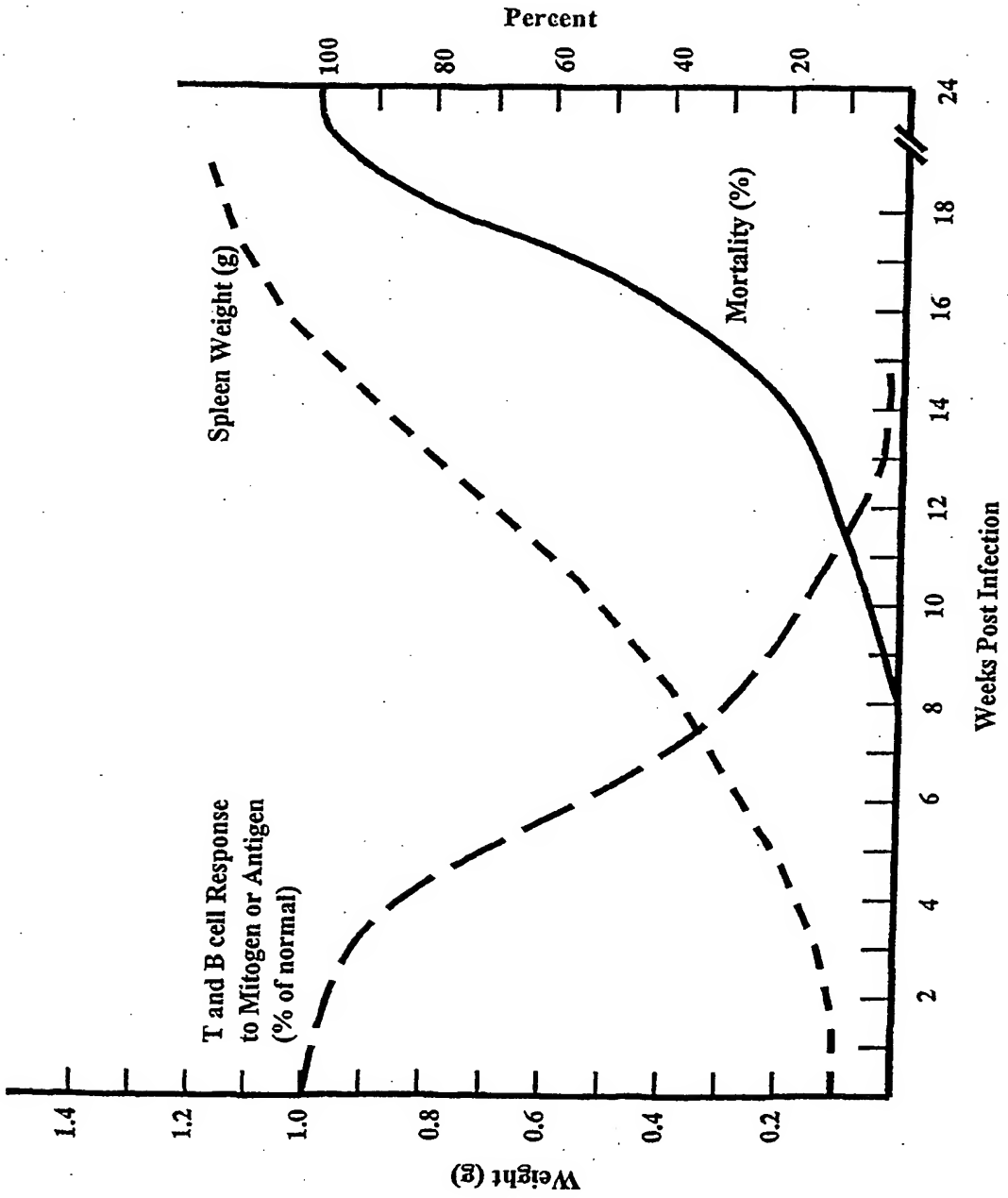
Percent
100
80
60
40
20
Spleen Weight (g)
Mortality (%)
T and B cell Response to Mitogen or Antigen (% of normal)
Weight (g)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
2
4
6
8
10
12
14
16
18
24
Weeks Post Infection

Figure 1.

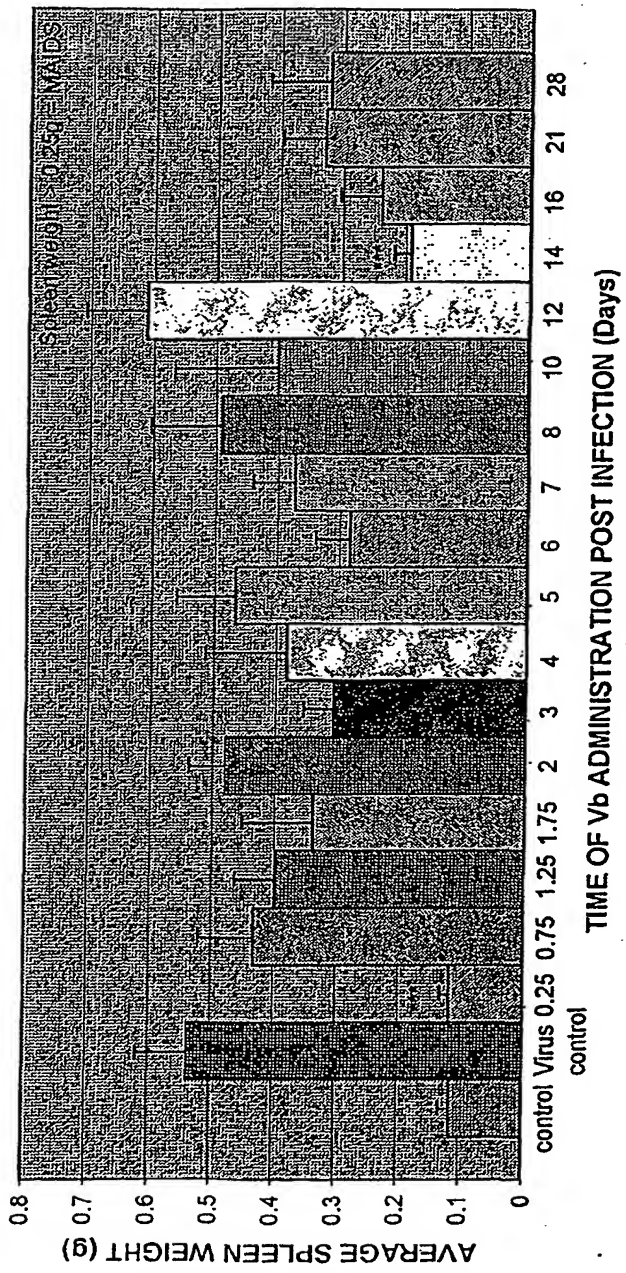
AVERAGE SPLEEN WEIGHT (g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
control
Virus control
0.25
0.75
1.25
1.75
2
3
4
5
6
7
8
10
12
14
16
21
28
TIME OF Vb ADMINISTRATION POST INFECTION (Days)

Figure 2.

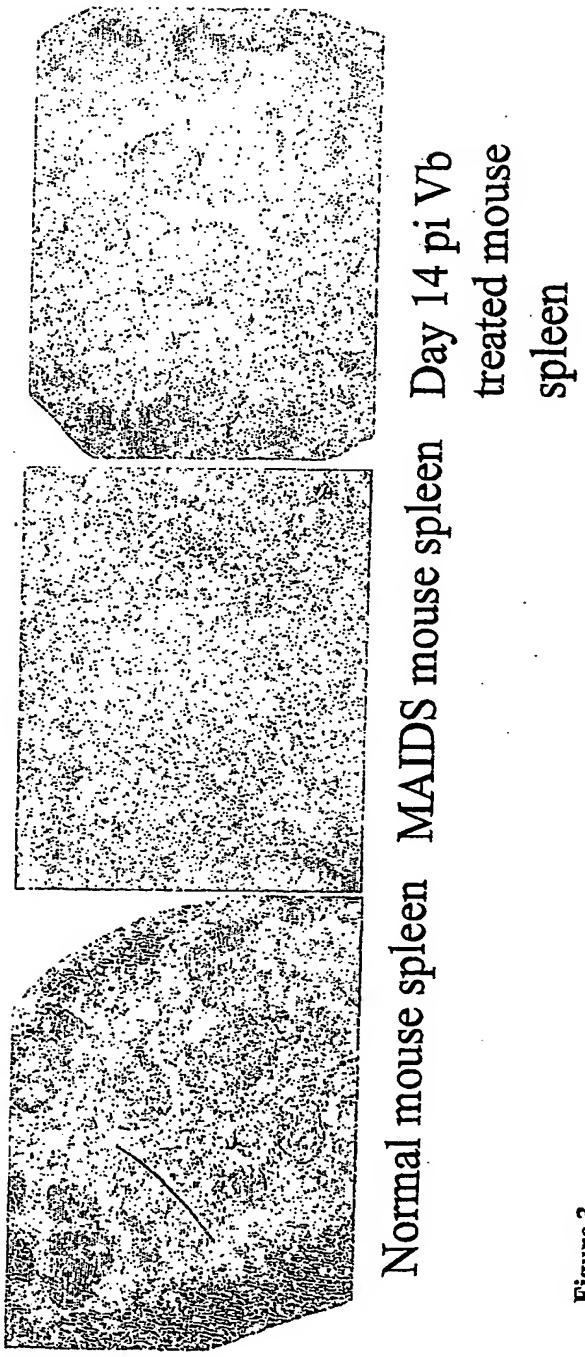
Normal mouse spleen
MAIDS mouse spleen
Day 14 pi Vb treated mouse spleen

Figure 3.

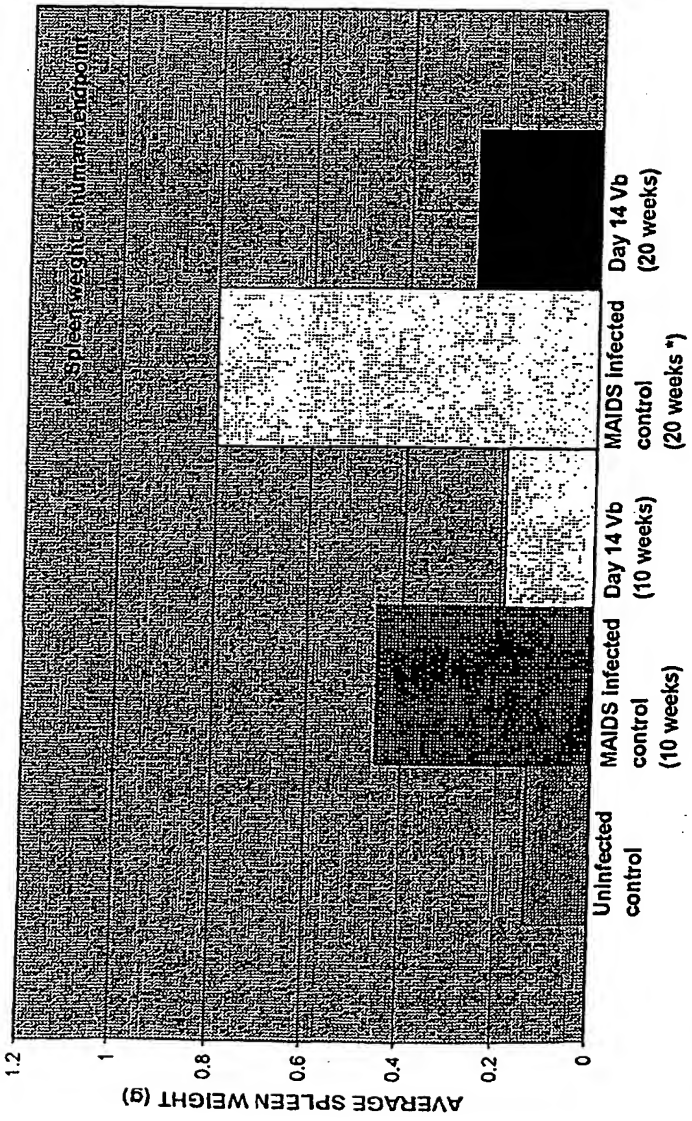
Spleen weight (mean ± SD)
AVERAGE SPLEEN WEIGHT (g)
1.2
1
0.8
0.6
0.4
0.2
0
Uninfected control
MAIDS infected control (10 weeks)
Day 14 Vb (10 weeks)
MAIDS infected control (20 weeks *)
Day 14 Vb (20 weeks)

Figure 4.

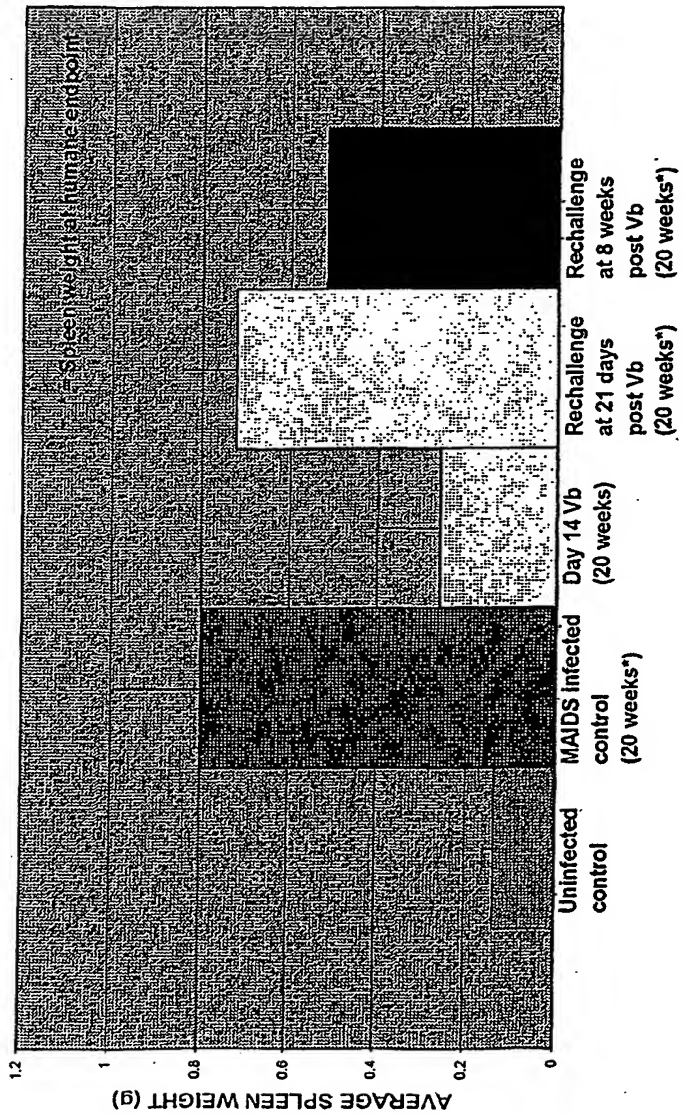
Spleen weight at humane endpoint
AVERAGE SPLEEN WEIGHT (g)
1.2
1
0.8
0.6
0.4
0.2
0
Uninfected control
MAIDS infected control (20 weeks*)
Day 14 Vb (20 weeks)
Rechallenge at 21 days post Vb (20 weeks*)
Rechallenge at 8 weeks post Vb (20 weeks*)

Figure 5.

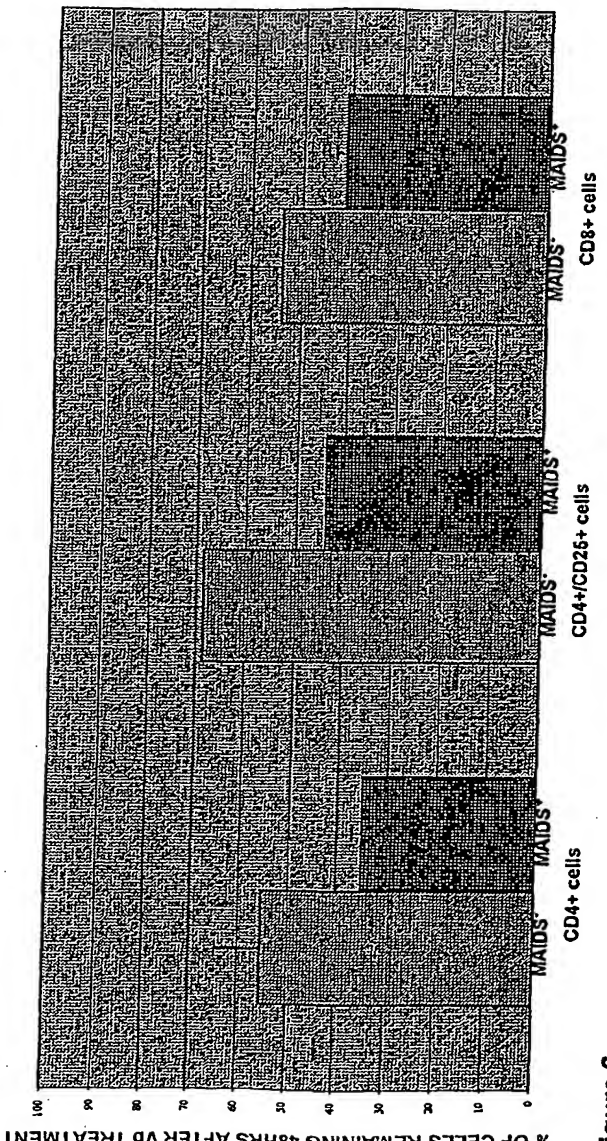
MAIDS+
MAIDS-
CD8+ cells
MAIDS+
MAIDS-
CD4+/CD25+ cells
MAIDS+
MAIDS-
CD4+ cells
% OF CELLS REMAINING 48HRS AFTER Vb TREATMENT

Figure 6.

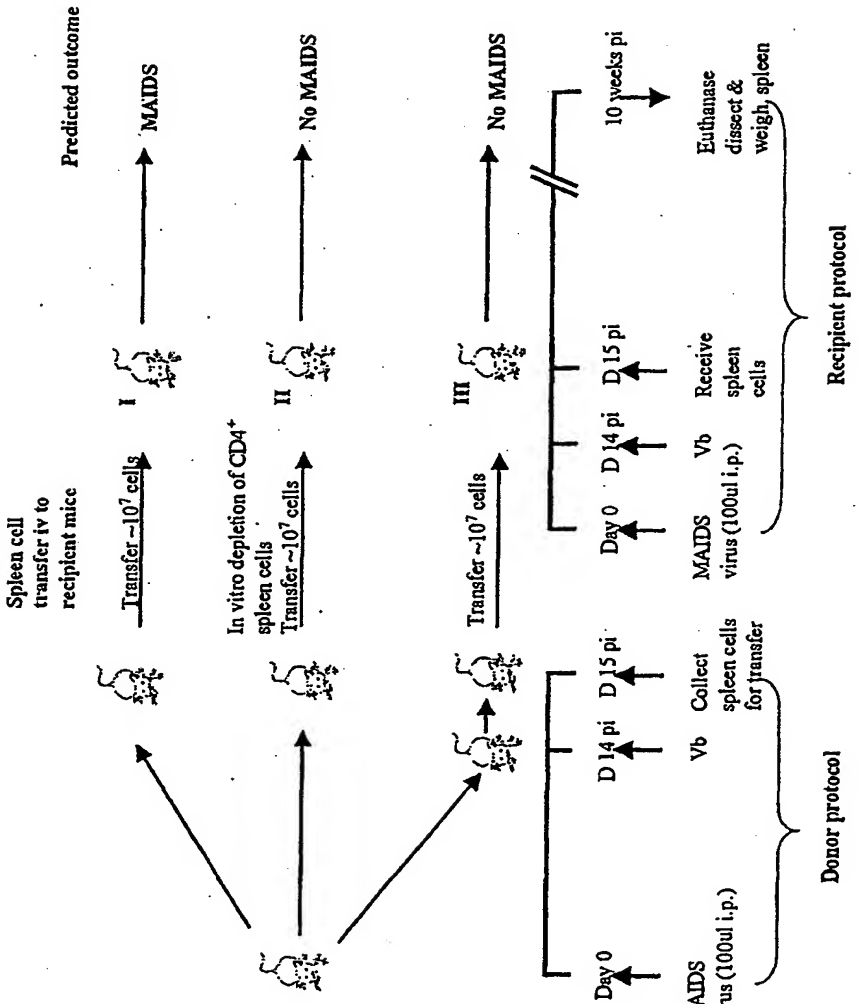
Predicted outcome
Spleen cell transfer iv to recipient mice
Transfer ~10⁷ cells
I
MAIDS
In vitro depletion of CD4⁺ spleen cells
Transfer ~10⁷ cells
II
No MAIDS
Transfer ~10⁷ cells
III
No MAIDS
Day 0
MAIDS virus (100ul i.p.)
D 14 pi
Vb
D 15 pi
Collect spleen cells for transfer
Donor protocol
Day 0
MAIDS virus (100ul i.p.)
D 14 pi
Vb
D 15 pi
Receive spleen cells
10 weeks pi
Euthanase dissect & weigh spleen
Recipient protocol

Figure 7.

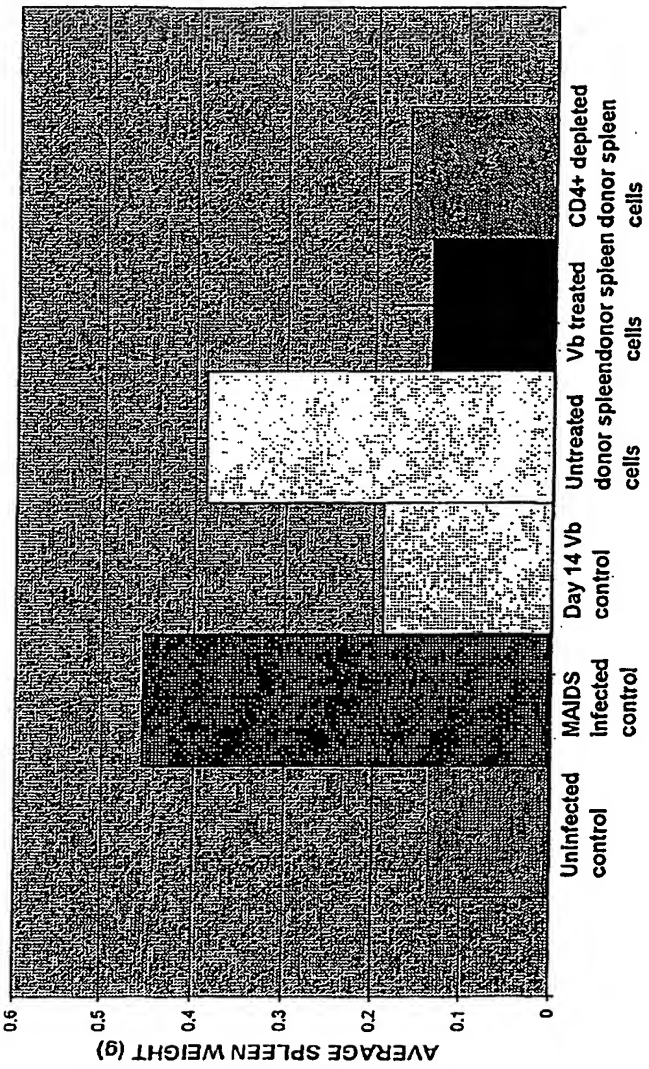
AVERAGE SPLEEN WEIGHT (g)
0.6
0.5
0.4
0.3
0.2
0.1
0
Uninfected control
MAIDS infected control
Day 14 Vb control
Untreated donor spleen cells
Vb treated donor spleen cells
CD4+ depleted donor spleen cells

Figure 8.

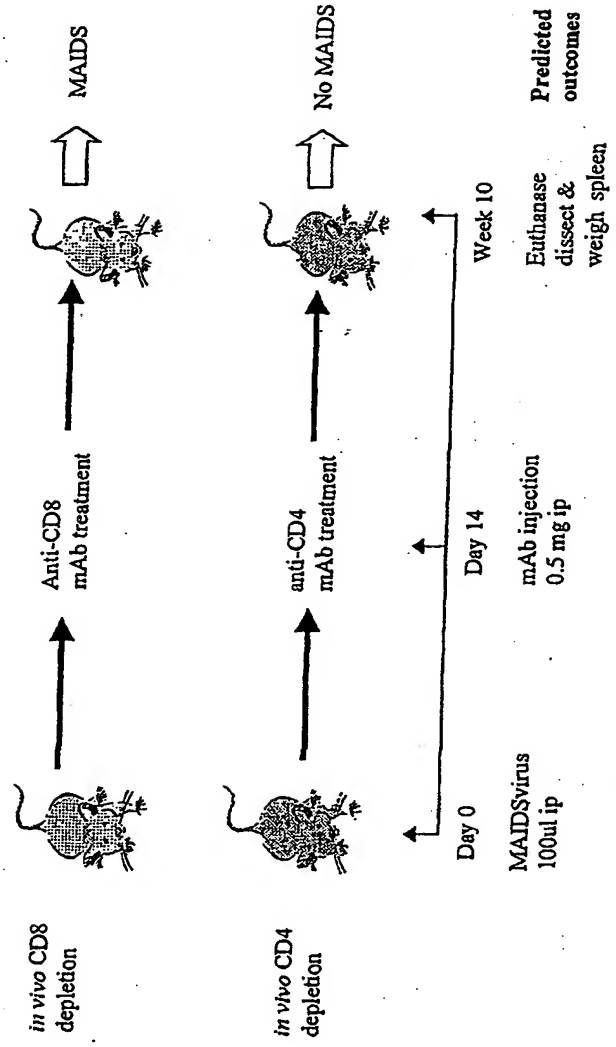
MAIDS
No MAIDS
Predicted outcomes
Anti-CD8 mAb treatment
anti-CD4 mAb treatment
Euthanase dissect & weigh spleen
Week 10
Day 14
mAb injection 0.5 mg ip
Day 0
MAIDSvirus 100ul ip
in vivo CD8 depletion
in vivo CD4 depletion
Controls received no monoclonal antibody treatment

Figure 9.

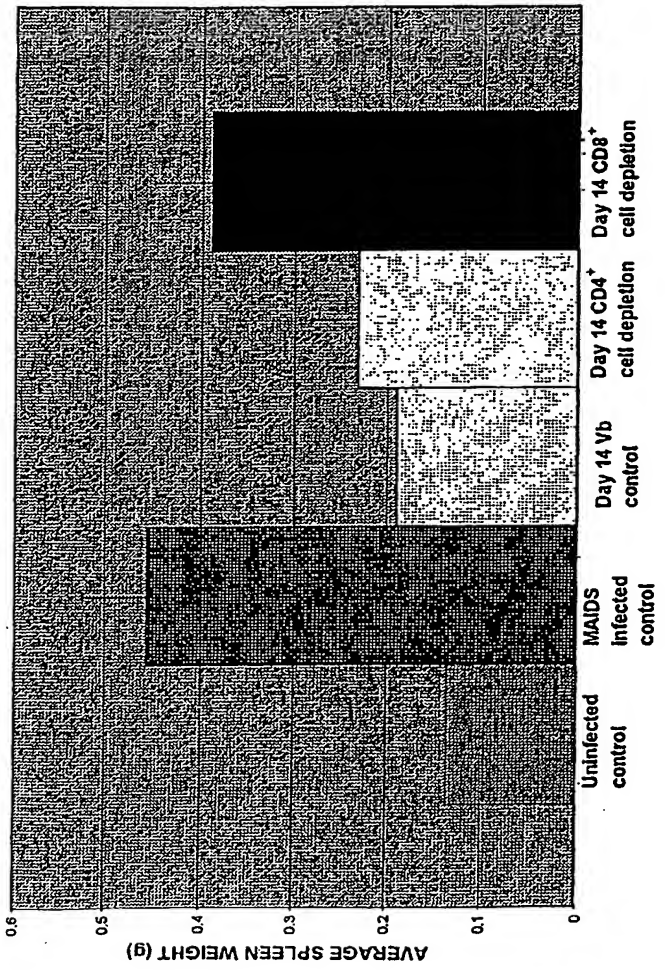
AVERAGE SPLEEN WEIGHT (g)
0.6
0.5
0.4
0.3
0.2
0.1
0
Uninfected control
MAIDS infected control
Day 14 Vb control
Day 14 CD4+ cell depletion
Day 14 CD8+ cell depletion

Figure 10.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01019

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 31/475, 39/21, A61P 31/18, 37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
REFER ELECTRONIC DATABASE CONSULTED BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DWPI, MEDLINE: IPC A61K & keywords: immundeficency, hiv, aids, retrovir, vinblastine, vincaleukoblastine, regulator cells, anti-prolifer, radiation, CD4, CD8, antibod

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Medline Abstract Accession No. PMID 10950369, Dezube BJ, *Semin Oncol*, August 2000, 27(4), 424-430	
X	abstract	18-19
A	abstract	1-17
	Medline Abstract Accession No. PMID 10228499, Aversa SM *et al.*, *Tumori*, Jan-Feb 1999, 85(1), 54-59	
X	abstract	18-19
A	abstract	1-17
	Medline Abstract Accession No. PMID 10093688, Errante D *et al.*, *Ann Oncol*, February 1999, 10(2), 189-195	
X	abstract	18-19
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Date of the actual completion of the international search
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International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Medline Abstract Accession No. PMID 10348092, Nasti G *et al.*, *Drug Saf*, May 1999, 20(5), 403-425	
X	abstract	18-19
A	abstract	1-17
	Medline Abstract Accession No. PMID 8959247, Zanussi S *et al.*, *AIDS Res Hum Retroviruses*, 10 December 1996, 12(18), 1703-1707	
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A	abstract	1-17
	Medline Abstract Accession No. PMID 2145216, North RJ *et al.*, *Immunology*, September 1990, 71(1), 90-95	
A	abstract	1-19